Carbon Isotope Ratios on Mars

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Objective:

To search for evidence of past or present life on Mars by looking for organic molecules in the Martian soil, and to measure precise carbon isotope ratios using the GC/MS method.

Background: Biosynthesis and Carbon Isotope Fractionation

Isotope ratios:

Analyzing the ratio of ^{13}C to ^{12}C in organic molecules provides clues to the processes that led to their formation. During CO_2 processing life tends to favor ^{12}C over ^{13}C, so organic compounds that are products of biosynthesis will have a different carbon isotope ratio than those that are created abiotically. The amount of isotopic fractionation depends on the specific processes that are used.

CO_2 Fixation and the Calvin Cycle:

CO_2 fixation(the incorporation of CO_2 into cell carbon) can occur along one of four known pathways: the rTCA cycle, the reductive acetyl CoA pathway, the 3-hydroxypropionate cycle, and the Calvin cycle. The Calvin cycle(Fig. 1) is used by plants as well as other autotrophs(such as cyanobacteria), and is by far the most common method of CO_2 fixation.

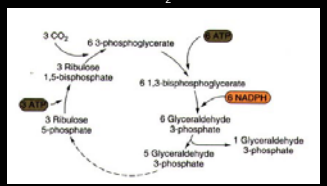

Fig. 1: The Calvin cycle. The first step is carbon fixation, in which CO_2 is combined with ribulose biphosphate(RuBP) to produce two 3-carbon compounds. The second step is reduction, in which these two molecules are reduced using energy stored in ATP and NADPH. Eventually fructose is produced, which is used for cellular metabolism. The third step is the regeneration of RuBP for another cycle.

Photosynthesis:

$^{13}C/^{12}C$ ratios in plants depend on the type of photosynthesis that takes place. There are three different pathways for photosynthesis: C3, C4, and CAM. C3 plants, so named because the first stable organic molecule formed has three carbons, put CO_2 directly into the Calvin cycle. C4 plants do an extra step before the Calvin cycle, and have enzymes that turn CO_2 into a 4-carbon molecule. CAM(crassulacean acid metabolism) plants can utilize both C3 and C4 processes for CO_2 fixation.

Why is all this important?

The metabolic pathway that an organism uses will determine the amount of carbon isotope fractionation that occurs. Also, the specific enzymes used in any of these processes have an effect on the isotope ratio. It is necessary to know typical $\delta^{13}C$ values for different types of organisms on Earth, so that we will have a idea of the kinds of variations we should be searching for on Mars. Typical isotopic fractionations for the above pathways are shown in Table 1:

Table 1. Overall Fractionations Associated with Carbon Fixation[a]

Pathway	$\delta^{13}C$(‰)
C3	10-22
C4 & CAM	2-15
Acetyl-CoA	15-36
rTCA	4-13

[a]adapted from Hayes (2001)

Clearly, there is a range of $\delta^{13}C$ values that can indicate the presence of life. Even if photosynthesis did not occur on Mars, methanogens could have used other such cycles to fractionate carbon. In fact, methanogens produce the largest $^{13}C/^{12}C$ fractionations on Earth – sometimes up to 70-100‰. However, the natural ratio of $^{13}C/^{12}C$ is 1.1%, so even if unusually high $\delta^{13}C$ values were expected, variations of less than about 0.05% would have to be measured in order to detect the effects of life.

2009: Mars Science Laboratory

The Mars Science Laboratory is a robotic mission scheduled for launch in 2009 that will explore and assess a local region on Mars as a potential habitat for life. The MSL will be a rover platform that will carry various payload instruments to at least three geologically different sites within one Martian year(687 days).

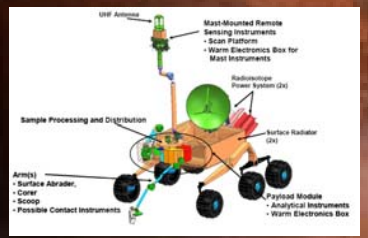

Fig 2: MSL fully deployed rover

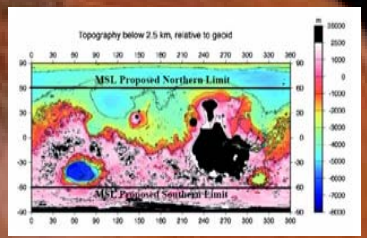

Fig 3: MSL landing site constraints.

The sub-objectives of the mission are to assess the biological potential of a specific habitat, study the geology of the landing region, study life-related processes such as the past water cycle, and to learn about the radiation environment at the surface. A gas chromatograph/mass spectrometer(GC/MS) is proposed to fly on the MSL as one of the payload instruments, and its purpose will be to detect trace amounts of organic molecules in soil samples and to determine isotope ratios.

Instrumentation: GC/MS

Derivatization Method:

Using pyrolysis techniques, the Viking MS experiments concluded that there are no detectable organics in the upper surface layer of Mars. This is likely inaccurate because organic molecules are not volatile and would not vaporize at the relatively low temperatures that were applied. In this case, derivatization is a better method of extraction, and will be used on the MSL.

Derivatization involves dissolving chemicals from a soil sample into a liquid, then applying a derivatizing agent to make the compound more volatile. Organic compounds contain labile H atoms, which tend to form hydrogen bonds that prevent the material from vaporizing. Replacing these H atoms with Si atoms makes the molecules less reactive, and then heating up the liquid only slightly will cause the organics to vaporize without being destroyed. Derivitazation makes it possible to detect extremely tiny amounts of organics in a sample.

GC/MS:

The gas chromatograph separates a mixture of gas into its constituents, producing a graph of peak intensity versus relative time. Different compounds exit the GC at different times, creating distinct peaks which are analyzed separately by the MS. The MS uses Electron Impact ionization to produce molecular and fragment ions, which are then separated by mass.

The instrument we use is a quadrupole mass analyzer(Fig. 4), which consists of four parallel rods that have fixed DC and alternating RF potentials applied. Ions are able to traverse the area between the rods, and as the potentials are varied sinusoidally with time, the ions in the central region follow complicated trajectories. The specific path an ion follows depends on its m/z, and varying the voltage combinations creates a stable path to the detector for certain ions. All others will hit the quadrupoles and will not be detected

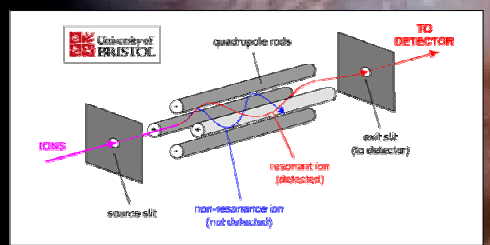

Fig. 4: Schematic of a quadropole mass analyzer

The end result is a mass spectrum: a plot of abundance versus m/z for a given compound. Molecular ion peaks will be present, as well as fragment ion peaks. Mass spectra can be used to determine the chemical composition of the gas and to detect isotope ratios.

Analyzing Mass Spectra

Fig. 5 shows an example of a mass spectrum for derivatized propanoic acid($C_{16}H_{37}NO_2Si_2$, molecular weight=331). The base peak is the highest peak in the spectrum, and abundances are scaled relative to it. The smaller peaks next to the larger ones are caused by the presence of isotopes.

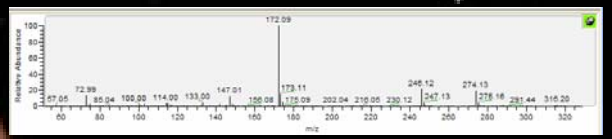

Fig 5: Mass spectrum of derivitized propanoic acid(MW=331). The base peak is at m=172, and there are other noticeable fragments at m=73, 147, 246, and 274.

Fragmentation and Isotope Ratios:

Derivatized organic molecules have predictable fragmentation patterns, and the expected relative intensity of isotope peaks can be found by knowing the chemical formula of the fragment(Fig. 6). The $^{13}C/^{12}C$ ratio can be found to a certain accuracy(depending on isotopic abundances of other elements in the fragment) by comparing the intensities of adjacent peaks(Fig. 7, Table 2).

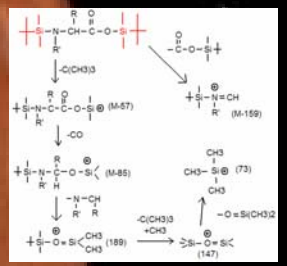

Fig. 6: Examples of some common fragmentation patterns. The derivitazation agent is shown in red.

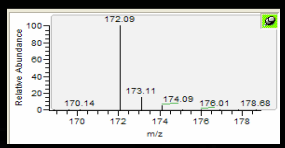

Fig. 7: Peak at 172 amu and the isotope peaks at 173 and 174 amu. This is the M-159 fragment ($C_9H_{22}NSi$), formed as shown in Fig. 6.

Table 2. Expected vs. observed isotope ratios

Peak	m=172	m=173	m=174
Expected	100	15.77	4.42
Observed	100	15.33	5.87
% difference		2.79%	32.8%

Error analysis:

Each GC peak is composed of many mass spectra, and observed isotopic abundances should be constant for peaks of a given mass. Standard deviation can be calculated by comparing isotopic peak ratios in mass spectra of the same compound. We have analyzed one simple hydrocarbon sample and two derivatized amino acid samples, and found that standard deviation drops steadily with increased count rate(Fig. 8).

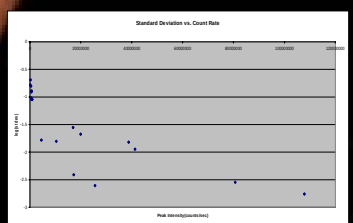

Table 3. Standard deviation

Sample	Max counts/s	Lowest stdev
1	9.11e5	9%
2	4.08e7	1.1%
3	1.08e8	0.18%

Fig 8: Standard deviation vs. count rate for all three samples

To detect changes in $\delta^{13}C$ as small as 0.05%, an extremely concentrated sample will be needed. We are currently working to determine the minimum concentration necessary for our GC/MS instrument to be able to detect the effects of life on the carbon isotope ratio. Also, we will be testing the MSL GC/MS prototype with noble gas samples to determine the precision of the new instrument.

*We gratefully acknowledge the support of the Delaware Space Grant Consortium.